Normocytic Anemia

Introduction

This lesson has a lot in it. All of it is very relevant. The first thing we do is discuss intravascular versus extravascular hemolysis and how you can use the urine as a clue to distinguish between the two. Some diseases have both intravascular and extravascular components, so this is not a great method for categorizing hemolytic anemias. Therefore, we then present our categorization of hemolytic anemias. This categorization is merely a cataloging exercise. Clinical practice comes down to recognizing that there is a hemolytic anemia, then using the blood smear to determine which is it. As we go through each of hemolytic anemias, we will provide the pathogenesis, presentation, and typical blood smear associated with it. We ran through blood smears in General #2: *Laboratory Interpretation*. Now we tie those blood smears to diseases.

Intravascular vs. Extravascular Hemolysis

The life span of a red blood cell is around **120 days**. That number is important because, through poorly understood mechanisms, around day 120 age-dependent changes in the red cell surface protein mark them for recognition and phagocytosis. The physiologic destruction of senescent red blood cells takes place within macrophages abundant in the spleen, liver, and bone marrow, which are together the reticuloendothelial system. This is all supposed to happen, and is normal.

Hemolytic anemias are defined by a shorted RBC life span to **fewer than 120 days**, a compensatory increase in erythropoiesis from an **elevated erythropoietin**, and the accumulation of **hemoglobin degradation products** that are created in the process of RBC hemolysis. Shortened life span, elevated EPO, and accumulation of degradation products define hemolytic anemia. But which "degradation product" accumulates depends on where the hemolysis occurs.

When the reticuloendothelial system does to young RBCs what it does to old RBCs (phagocytosis), it is deemed **extravascular hemolysis**. Extravascular hemolysis is "intra-reticuloendothelial" hemolysis. The unifying characteristic of the spleen, liver, and bone marrow is **sinusoids**, capillaries with large gaping holes between endothelial cells where macrophages lie in wait. To move through these small blood vessels, RBCs must deform. If they can't deform, they won't fit through the sinusoids, and they'll get stuck. If they get stuck, the macrophages phagocytose them. The spleen is the major site of this process, and clearing these RBCs is the main function of the red pulp of the spleen. When chronic and ongoing, extravascular hemolysis results in the hyperplasia of phagocytes manifested as **splenomegaly**. The macrophages phagocytose RBCs, degrading hemoglobin to heme and iron, storing the iron as hemosiderin or releasing it onto transferrin. Macrophages release unconjugated bilirubin into the bloodstream, which binds to albumin and is taken to the liver for conjugation and elimination. Excess bilirubin causes **jaundice**. Because the RBCs are being destroyed prematurely, there is **anemia**. Therefore, extravascular hemolysis is characterized by anemia, jaundice, and splenomegaly. Because the lysis of the RBC is within the macrophage, very little free hemoglobin is released into the bloodstream. Whatever free hemoglobin is released is bound by **haptoglobin**, preventing hemoglobin from being passed in the urine.

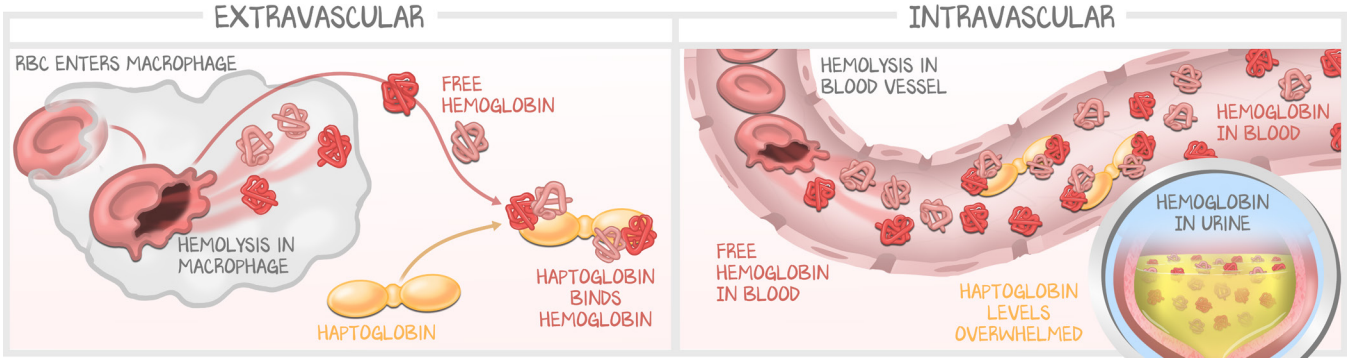


Figure 7.1: Extravascular vs. Intravascular Hemolysis
(a) Extravascular hemolysis is the destruction of red blood cells by macrophages within the reticuloendothelial system. Little free hemoglobin is released from the macrophage, and all that is gets bound to haptoglobin. No hemoglobin ends up in the urine. (b) Intravascular hemolysis is the lysis of red blood cells not contained in macrophages, releasing excess hemoglobin into the bloodstream that overwhelms haptoglobin levels, leading to hemoglobin in the blood and urine.

Intravascular hemolysis, in contrast, is what happens when the RBC explodes, lyses not within a macrophage, but free-floating in the vessels. The causes of intravascular hemolysis tend to be trauma, complement fixation, and intracellular parasites such as malaria or babesia. Most hemolytic anemias are extravascular. Those that are intravascular present with **anemia** and **jaundice**, just like extravascular hemolytic anemias—haptoglobin hemoglobin complexes are phagocytosed by macrophages, leading to the release of unconjugated bilirubin. But because there is no splenic phagocytosis of RBCs, there is no splenomegaly. And because there is no systematic breakdown of hemoglobin within a lysosome of a macrophage, a lot of hemoglobin is released into the plasma. Whatever haptoglobin there is binds to hemoglobin, preventing it from being passed in the urine. But there is only enough haptoglobin to accommodate the small amount of hemoglobin released during phagocytosis and digestion. With intravascular hemolysis, excess hemoglobin ends up in the urine. Intravascular hemolysis is therefore manifested as **anemia, jaundice, hemoglobinemia** (in the blood), and **hemoglobinuria** (in the urine). As serum haptoglobin is depleted, free hemoglobin oxidizes to methemoglobin, which is brown in color. The renal tubules absorb and process the methemoglobin and hemoglobin, but some is lost to the urine, turning the urine brown.

Overarching Causes of Hemolytic Anemia

The first branch-point in the pathogenesis of hemolytic anemias is whether there is a **defect of the RBC** (intrinsic hemolytic anemia) or whether something has been **done to the RBC** (extrinsic hemolytic anemia). Since there are so many hemolytic anemias, this is useful only to help keep them sorted. **Intrinsic** hemolytic anemia is further subdivided into problems with hemoglobin (hemoglobinopathies), dysfunction of the plasma membrane or cytoskeleton, and enzyme deficiencies (metabolic). **Extrinsic** hemolytic anemias take normal, healthy RBCs that would have otherwise lived a full life, and destroy them too soon.

There was a long discussion of intravascular versus extravascular hemolysis in the preceding section. That is a much better way of categorizing diseases based on the patient presentation—jaundice, hemoglobinemia, or hemoglobinuria vs. none of those things. However, it is also potentially confusing. Many of the diseases we are going to discuss have both an intravascular and extravascular component. Further, intravascular and extravascular is one categorization. Intrinsic and extrinsic is another. There is no overlap of the two categorizations. Because all hemolytic anemias are either intrinsic OR extrinsic (and never both), and because some hemolytic anemias CAN be both intravascular and extravascular, we choose to organize the remainder of this lesson by intrinsic vs. extrinsic.

INTRINSIC			EXTRINSIC		
Hemoglobinopathies	Membrane/ cytoskeleton	Metabolic	Mechanical/ traumatic	Antibody mediated	Infection
Sickle cell disease	Hereditary spherocytosis	G6PD deficiency	Microangiopathic hemolytic anemia	Cold AIHA	Malaria
HbC	PNH	Pyruvate kinase deficiency	Macroangiopathic	Warm AIHA	Babesia

Table 7.1: Preview and Categorization of Hemolytic Anemias

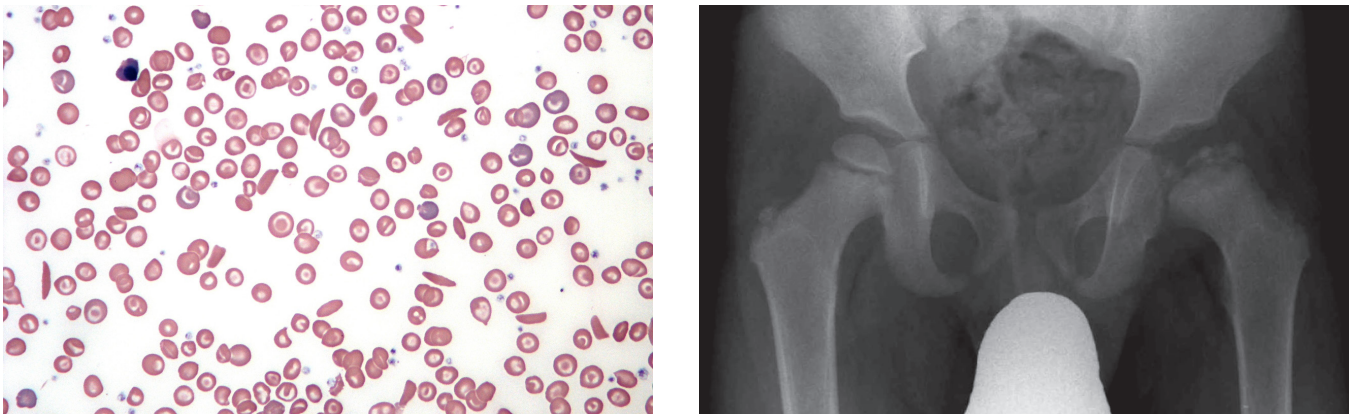
Intrinsic—Hemoglobinopathies

Sickle cell disease. There is a lot to know about sickle cell disease. We are going to take an abbreviated approach to this disease, going for breadth rather than depth in each subject. Sickle cell is an **autosomal recessive** mutation of the β-globin gene. It requires two copies to provoke symptoms. The heterozygous state has been shown to provide protection against malaria, a disease of Africa. Therefore, in the United States, **African Americans** demonstrate the high prevalence of both sickle cell trait (heterozygous) and sickle cell disease. The mutation is a **point mutation** in the **7th position** of chromosome 11, making the normal **glutamate** into the pathologic **valine**. HbS, in periods of hypoxemia or acidosis, **irreversibly polymerizes** within an RBC, forming the **sickled cell**. Sickled cells cannot deform, and result in two outcomes. The first is **occlusion** of vessels. This leads to **vaso-occlusive crises** (joint pain, acute chest, stroke, priapism) that punctuate a patient's life. Occlusion also leads to infarction. Infarction causes **avascular necrosis of the hip** and **autosplenectomy**. The second is hemolysis. Sickled cells cannot fit through the sinusoids of the reticuloendothelial system, and are destroyed. In childhood, the spleen acts as a source of extravascular hemolysis. **Splenomegaly is common in children.** By adolescence, the spleen has been infarcted so severely that they are functionally asplenic. From the diagnosis to the age of 18, all children with sickle cell disease are given **prophylactic penicillin** and must subsequently be vaccinated against encapsulated organisms.

In anyone without a spleen, hemolysis is both intravascular and extravascular.

The initial diagnosis is made by finding sickled cells on smear. A confirmatory one-time **hemoglobin electrophoresis** confirms HbS. The patient will always be in a state of chronic hemolysis—the bilirubin will be elevated, the haptoglobin will be low, and the reticulocyte count will be elevated. The patients live at a hemoglobin of around 7. During periods of acute hemolysis, during a vaso-occlusive crisis, the bilirubin will rise from baseline, the reticulocytes will rise from baseline, and the hemoglobin will fall from baseline. **Sickled cells** on blood smear confirm acute attack. These patients often become **iron overloaded** because of routine transfusions. Iron chelation (**deferoxamine**) is usually indicated. In addition, because they are in a state of chronic hemolysis, synthesis of new RBCs maintains their hemoglobin. **Folate** supplementation prevents production abnormalities. **Aplastic crisis** from parvovirus B19 is of greatest risk in SCD.

Some unique features of sickle cell disease are commonly board-tested. Salmonella **osteomyelitis** is associated with sickle cell disease, rather than the usual *Staph. aureus* osteomyelitis. Because of chronic hemolysis and elevated bilirubin, these patients are at high risk for **pigmented gallstones**. In the case of vaso-occlusive crisis leading to stroke symptoms, MI symptoms (acute chest), or priapism, an **exchange transfusion** is required (pull out the sickled cells, give blood without the diseased hemoglobin). **Hydroxyurea** is used to shift the balance of diseased HbS and HbF. Normally, there is about 90% HbS and 10% HbF. With hydroxyurea, that shifts to 60% HbS and 40% HbF. Hemoglobin F, which does not rely on the β-gene and does not sickle, reduces crises and prolongs life.



(a) (b)

Figure 7.2: Sickle Cell Disease
(a) Blood smear showing sickled cells. (b) X-ray showing avascular necrosis of the hip.

Hemoglobin C. Also caused by a point mutation of the 7th amino acid of chromosome 11, the glutamate is turned into a **lysine**. This causes precipitation of hexagonal hemoglobin crystals in RBCs. It does not cause sickling. The combination of HbS and HbC (two defective genes, one with valine and one with lysine) has a more severe presentation than HbC, but a less severe presentation than having sickle cell disease.

Intrinsic—Membrane and Cytoskeleton

Hereditary spherocytosis. The plasma membrane is attached to the cytoskeleton, which is attached to more plasma membrane. Imagine the cytoskeleton with a thousand arms to a thousand handholds on the plasma membrane. This keeps the plasma membrane around the cell intact. If those handholds fail, small blebs of plasma membrane fall off. Young RBCs have their normal shape and normal complement of plasma membrane. As RBCs age, blebs of plasma membrane continue to fall away from the cell. The loss of membrane relative to the cytoplasm forces the cell to assume the smallest possible diameter for its volume: a sphere. In hereditary spherocytosis, any one of the molecules responsible for connecting the plasma membrane to the cytoskeleton fails—**band 3**, **ankyrin**, **spectrin**, and **protein 4.1**. Yes, those are the names of the proteins, and yes, you must know all four.

These RBCs are smaller than normal. They are smaller because they have lost plasma membrane, and more plasma membrane than they did cytoplasm within that bleb. That means they are smaller, but have just as much hemoglobin as they did when they were bigger. "Smaller size" combined with "same hemoglobin" means the concentration of hemoglobin is higher in the cell. Said differently, the MCV decreases and the MCH remains the same, which means the **MCHC is elevated** in HS. Likewise, the sphere shape lacks the central pallor characteristic of the biconcave RBCs; hence, the spherocyte is **dark red without pallor**. The shape of a sphere cannot be appreciated on a blood smear slide, so instead they appear as circles.

The ability to fit through the sinusoids of the spleen depends on the ability of the cell to distend. That is very possible when the shape is biconcave. It is impossible when the shape is a perfect sphere. Because the younger cells have normal cytoplasm and membrane, and because membrane blebs off over time, older spherocytes are phagocytosed. The life span of hereditary spherocytosis RBCs is 10–20 days shorter than normal RBCs. Because the spleen does most of the hemolysis, there is splenomegaly from extravascular hemolysis. **Splenectomy** can cure the anemia but will not cure the spherocytes. The disease

is an inherited defect, **autosomal dominant**, that exists within the erythrocyte progenitor. The spleen just does what it is supposed to do—remove old, broken RBCs. The problem is that what the spleen sees as old and broken is actually quite functional, except for its membrane problem. Diagnosis is made with an **osmotic fragility** test.

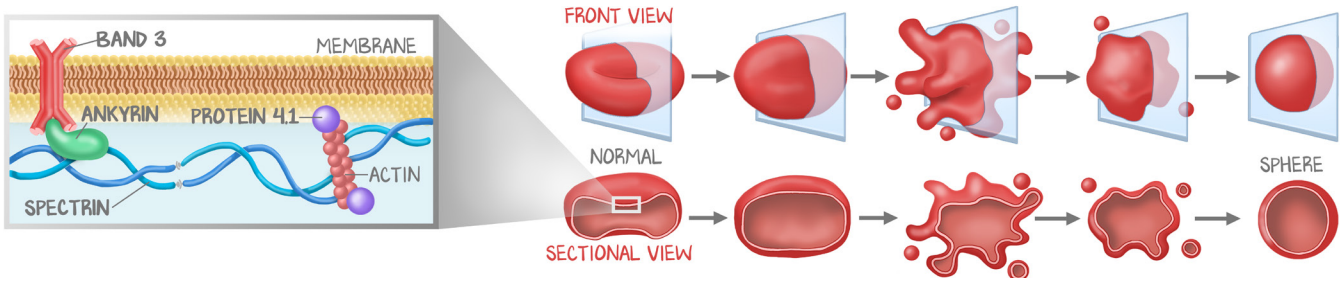


Figure 7.3: Hereditary Spherocytosis
Hereditary spherocytosis results from the loss of plasma membrane over time, forcing the cells to become a sphere. Loss of any of the proteins that link the cytoskeleton to the plasma membrane can result in spherocytes. Young red blood cells have a normal appearance. Older red blood cells assume the shape of the sphere.

Paroxysmal nocturnal hemoglobinuria. PNH results from a mutation in the phosphatidylinositol glycan complementation group A gene (**PIGA gene**). Some extracellular proteins stay attached to the plasma membrane by being IN the plasma membrane (transmembrane proteins). Others rely on **GPI anchors** (glycosyl-phosphatidyl-inositol). Without the GPI anchor, the extracellular proteins just float away. In PNH, an **X-linked recessive** disorder that causes a defect in the GPI anchor, patients are deficient in three GPI-linked proteins. The first is **CD55**, known as decay-accelerating factor, a potent inhibitor of C3 convertase that prevents spontaneous activation of the complement cascade. The second is **CD59**, which prevents the formation of the MAC attack complex (C5–C9). The third is C8-binding protein, which has no clinical significance. The diagnosis is made with **flow cytometry**, which results in a sample negative for CD55 and CD59.

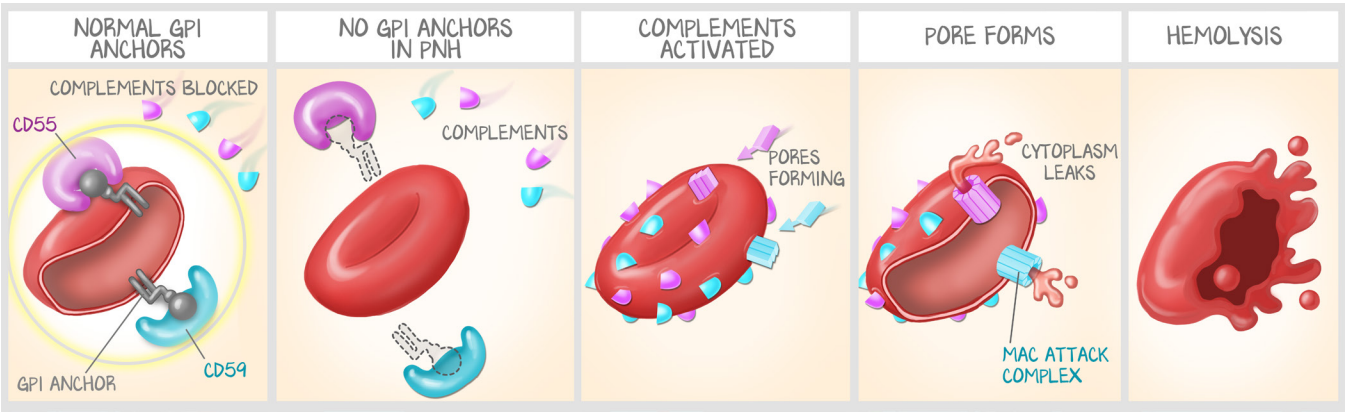


Figure 7.4: Paroxysmal Nocturnal Hemoglobinuria
PNH is caused by the loss of GPI anchors. Loss of those anchors results in the loss of the protective proteins CD55 and CD59, making red blood cells vulnerable to compliment fixation and intravascular hemolysis.

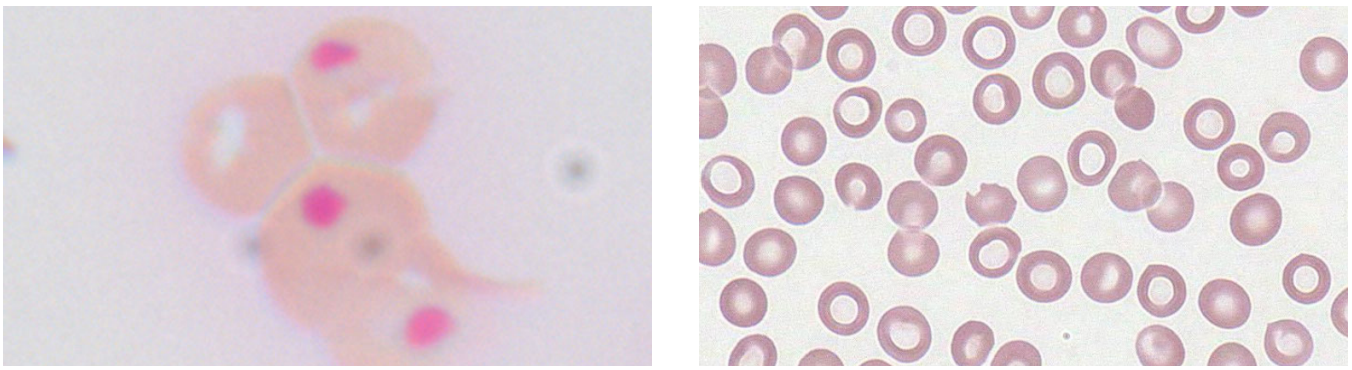
Under normal conditions, complement binds to RBCs. Decay-accelerating factor (CD55) prevents the complement cascade from continuing. Even if it does, CD59 prevents the formation of the MAC attack complex. In PNH, neither of these proteins is there, and the cell is vulnerable to complement. At night, **when respiration becomes shallow**, there is a relative **respiratory acidosis.** Acidosis promotes the activation of complement. **Intravascular** hemolysis occurs at night, resulting in the release of hemoglobin in the blood and into the urine. The person wakes up and urinates with **tea-colored hemoglobinuria**.

Even though the name of the disease is paroxysmal nocturnal hemoglobinuria (the dramatic and obvious presentation), most cases are in fact a **mild chronic** disease. With loss of hemoglobin into the urine comes loss of iron into the urine, which can lead to iron deficiency anemia in addition to PNH. The anemia is usually not severe enough to cause many problems. Complement can form on leukocytes, resulting in leukopenia, or on platelets. Lysis of platelets results in the release of dense bodies and α-granules, predisposing the patient to **thrombosis**.

Intrinsic—Metabolic

G6PD deficiency. Glucose-6-phosphate dehydrogenase is an enzyme that, as all dehydrogenases do, makes a high-energy compound. In this case, it makes NADPH. NADPH is required for glutathione to do its thing. "Its thing" is a convoluted pathway of reductions and oxidations and enzymes that have glutathione in their name. Do not learn that pathway. Learn instead that without G6PD, the RBC runs out glutathione and **cannot defend against oxidant stress**. RBCs don't have a nucleus or mitochondria. They can't synthesize new proteins. But they had mitochondria while they were maturing, and they made protein before they left the bone marrow. All variants of G6PD deficiency (of which there are hundreds) result in a **reduced amount of G6PD** expressed; no variant has an absence of G6PD. That means younger RBCs have G6PD and can resist oxidant stress. Older RBCs run out and cannot resist oxidant stress. Therefore, **old RBCs are the most vulnerable**. G6PD is not a chronic hemolytic anemia. Anemia occurs only during times when the patient is exposed to excess **oxidant stress**.

Oxidant stress comes in three forms—infection, drugs, and food. **Infections** cause oxidant stress because activated leukocytes make oxygen free radicals. **Drugs** include chloroquine (malaria prophylaxis), sulfonamides (TMP/SMX), nitrofurantoin, and dapsone. **Food** is classically represented by the fava bean. The patient will live normally, without anemia and without jaundice for most of their life. When they happen upon oxidant stress, they will experience **anemia and jaundice** as those older RBCs who are running out of G6PD undergo intravascular hemolysis. This occurs 2–3 days after the oxidant stress is encountered. The cells that don't die had G6PD enough to live, and so the remaining RBCs are unaffected. Therefore, the episode is **self-limiting**. Recovery is heralded by reticulocytosis.



(a) (b)

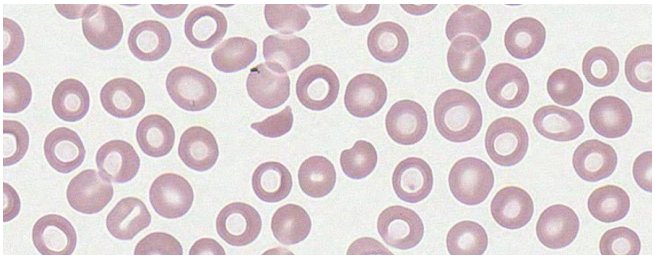
Figure 7.5: G6PD Deficiency
(a) Blood smear showing Heinz bodies, aggregations of hemoglobin with red blood cells. (b) Blood smear showing the result of a removed Heinz body, the bite cell.

Oxidant stress induces the cross-linking of sulfhydryl groups on globin, causing clumpings called **Heinz bodies**, which are seen as dark blue inclusions within red cells under special stains. Heinz bodies are what cause membrane damage and provoke intravascular hemolysis. However, those cells that develop Heinz bodies but do not lyse circulate through the spleen. There, Heinz bodies are plucked out by macrophages, leading to a small degree of extravascular hemolysis. The RBC is not phagocytosed, and what is left is the **bite cell**. Both Heinz bodies and bite cells are indicative of G6PD deficiency, but are not diagnostic. Assessment of G6PD levels will confirm the diagnosis. However, as the only surviving RBCs had G6PD, measuring during or near to the time of an episode of hemolysis will lead to a false negative. Measure G6PD levels several weeks after an episode.

Pyruvate kinase deficiency and **hexokinase deficiency** are other metabolic causes of hemolytic anemia. They are rare and don't have associated blood smears, so are not discussed in any detail.

Extrinsic—Trauma

Microangiopathic. For microangiopathic hemolytic anemia (MAHA), think **DIC/TTP** and **schistocytes**. Disseminated intravascular coagulation (DIC) forms fibrin clots. These fibrin clots consume platelets, clotting factors, and fibrinogen. The patient bleeds everywhere, despite thrombosis throughout the vasculature. When RBCs deform to pass through small blood vessels, they encounter these fibrin clots, have nowhere to go but into their jagged edges. Those that make a direct hit are hemolyzed. Those that barely slip by are sheared, forming **schistocytes.** In thrombotic thrombocytopenic purpura (TTP), platelet plugs form everywhere. Some may progress to form complete thrombosis. But everywhere an RBC needs to deform to fit through the vessel, these tiny microthrombi are waiting. Those RBCs that make a direct hit are hemolyzed. Those that bare slip by are sheared, forming **schistocytes**. DIC and TTP are both MAHAs so both cause anemia and their blood smears show schistocytes. DIC and TTP are also both life-threatening thrombotic and thrombocytopenic diseases. You will be responsible for seeing a schistocyte, anemia, and thrombocytopenia and deciding which disease is present. The discussion in this lesson was about the MAHA alone, about the formation of schistocytes. The details of TTP and DIC are covered in Clotting #4: *Platelet Bleeding*.



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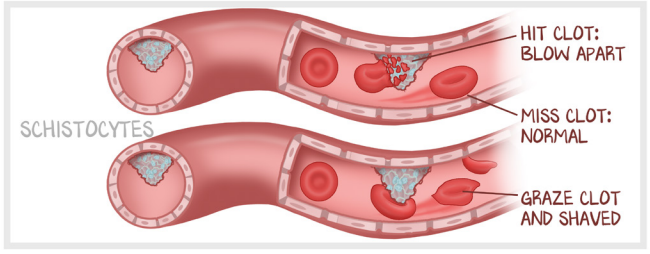

(b)

Figure 7.6: Microangiopathic Hemolytic Anemia (MAHA)
(a) Schistocytes on blood smear. (b) An illustration showing what schistocytes are in disseminated intravascular coagulation. A clot forms. Red blood cells that escape the trauma are left unaffected. Those that collide with the clot are hemolyzed. Those that graze the clot are sheared, forming schistocytes.

Macroangiopathic. You can get schistocytes from trauma, as seen in mechanical valves. So, while not every schistocyte means a life-threatening illness, we encourage you to use that as your starting point. If schistocytes, look for TTP and DIC. If not present, look for something else; oftentimes it will be an aortic valve.

Extrinsic—Antibody Mediated

The diagnosis of autoimmune hemolytic anemia (AIHA) requires the detection of antibodies or complement on RBCs. This is done with the Coombs test. We take sample of whole blood from the patient. We spin it down. We take out the RBCs for the direct Coombs test and the serum for the indirect Coombs test. The **direct Coombs** test involves mixing the patient's RBCs with commercially available serum containing antibodies that are specific for human Ig or complement. If there is complement or Ig on the patient's RBCs, the sample agglutinates. In the **indirect Coombs** test, the patient's serum, already known to have antibodies in it from the direct Coombs, is tested against commercially available RBCs, each sample of RBCs bearing a specific antigen. Multiple samples are run, each sample with the patient's serum and RBCs with a particular antigen. This test is used to characterize the antigen target and temperature dependence of the responsible antibody. In most samples, there is no agglutination. In the sample that agglutinates, since the RBCs are known to express specific antigens, the antigen is defined by whatever the RBCs have on them.

Cold AIHA. Cold hemolytic disease is caused by **IgM** antibodies and is associated with **m**ycoplasma and **m**ono. The function and activity of these antibodies and complement fixation requires colder temperatures than core body temperature, such as exposed fingers, toes, and ears. IgM facilitates the fixation of complement in vascular beds of these organs. When the blood circulates and rewarms, the IgM dissociates. The complement remains and acts as an opsonin for the macrophages in the spleen. Cold AIHA results in extravascular hemolysis. It is usually acute only, subclinical, and self-limiting. Chronic cold AIHA is seen with CLL.

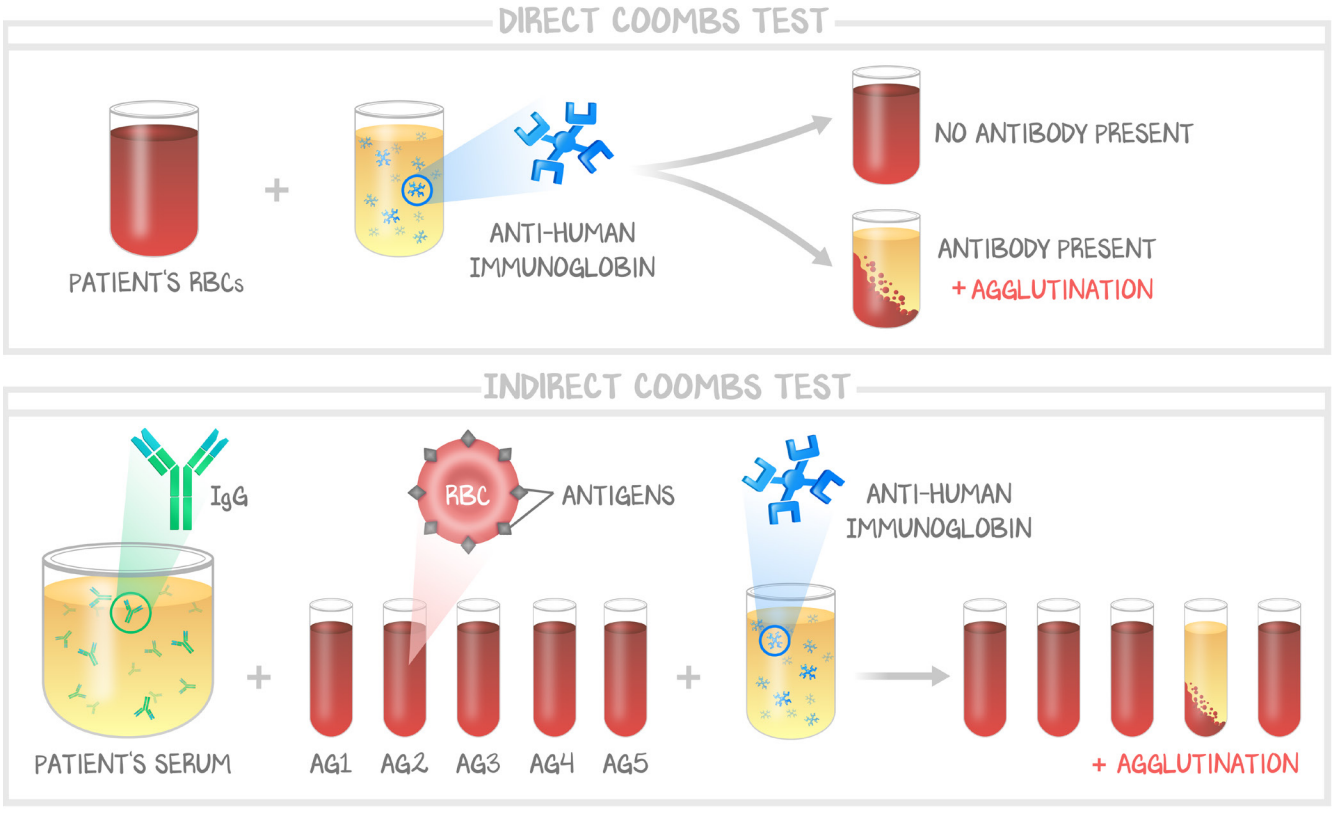

Figure 7.7: Coombs Test
In the direct coombs test, antibodies against the Fc portion are added to patients blood. This answers if the patient has antibodies to red blood cells in their blood at all. Agglutination means there are antibodies. Then the indirect Coombs test tests the patient's serum for which antibody is present. Serum is added to samples of blood with a known antigen in each sample. Agglutination means there is an antigen in that sample that the patient's autoantibody binds to.

Warm AIHA. Warm AIHA is caused by drug reactions (penicillin, α-methyldopa), autoimmune disease (lupus), cancer (manifesting as a paraneoplastic syndrome), or is considered idiopathic. **IgG** antibodies identify RBC membrane proteins. These IgG-coated plasma membranes circulate to the reticuloendothelial system. The fc portion of IgG does act as an opsonin, but induces only **partial phagocytosis**. The net effect is a reduction in plasma membrane relative to the cytoplasm. Does that phrase sound familiar? Just as in hereditary spherocytosis, RBCs form the shape of a sphere, which cannot deform, and get stuck. **Spherocytes** do not equate with hereditary spherocytosis, and that blood smear may well be warm AIHA.

Extrinsic—Infections

Malaria and babesiosis are discussed in Microbiology: Parasites #1: *Protozoa*. These represent an intravascular hemolysis.